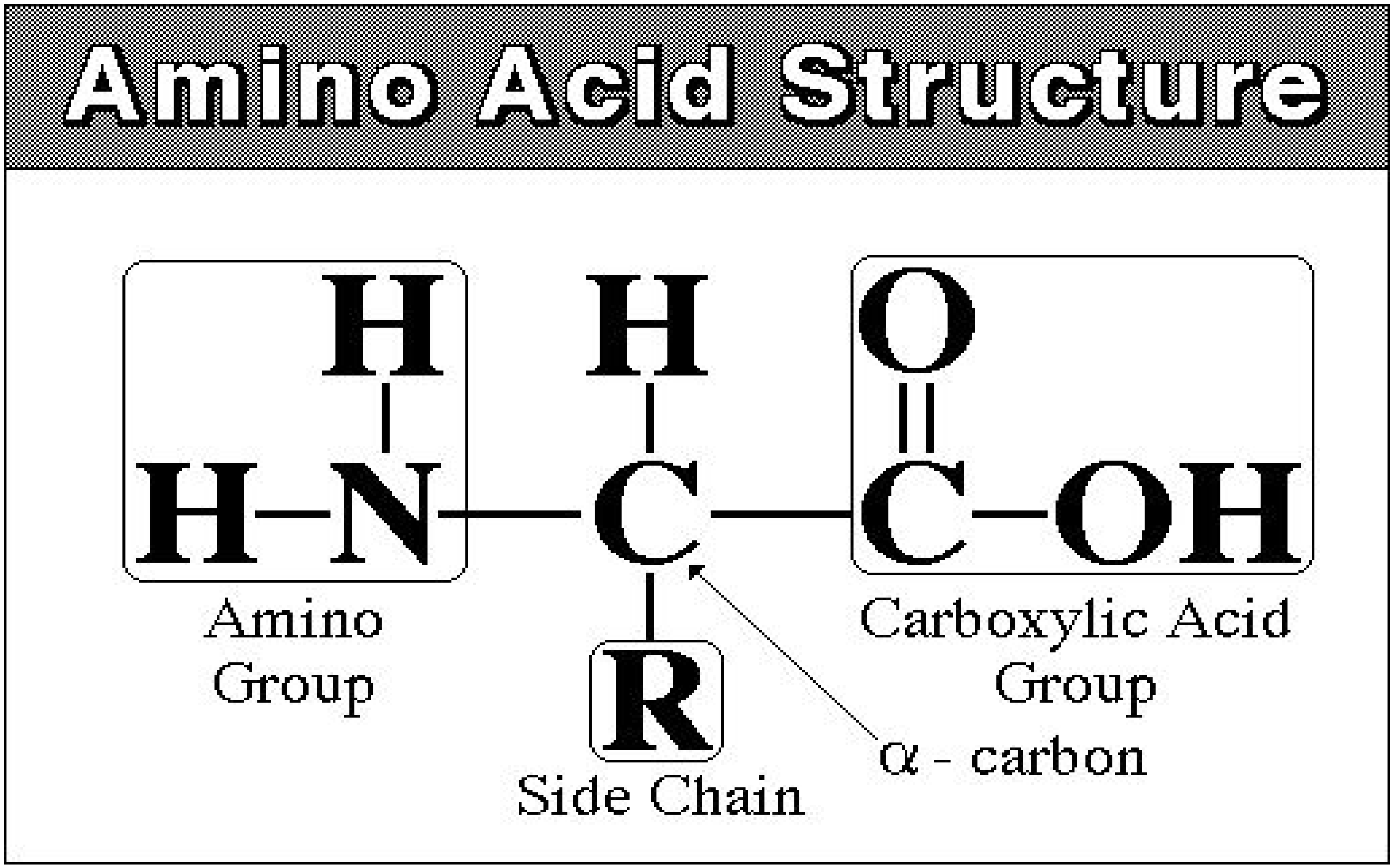
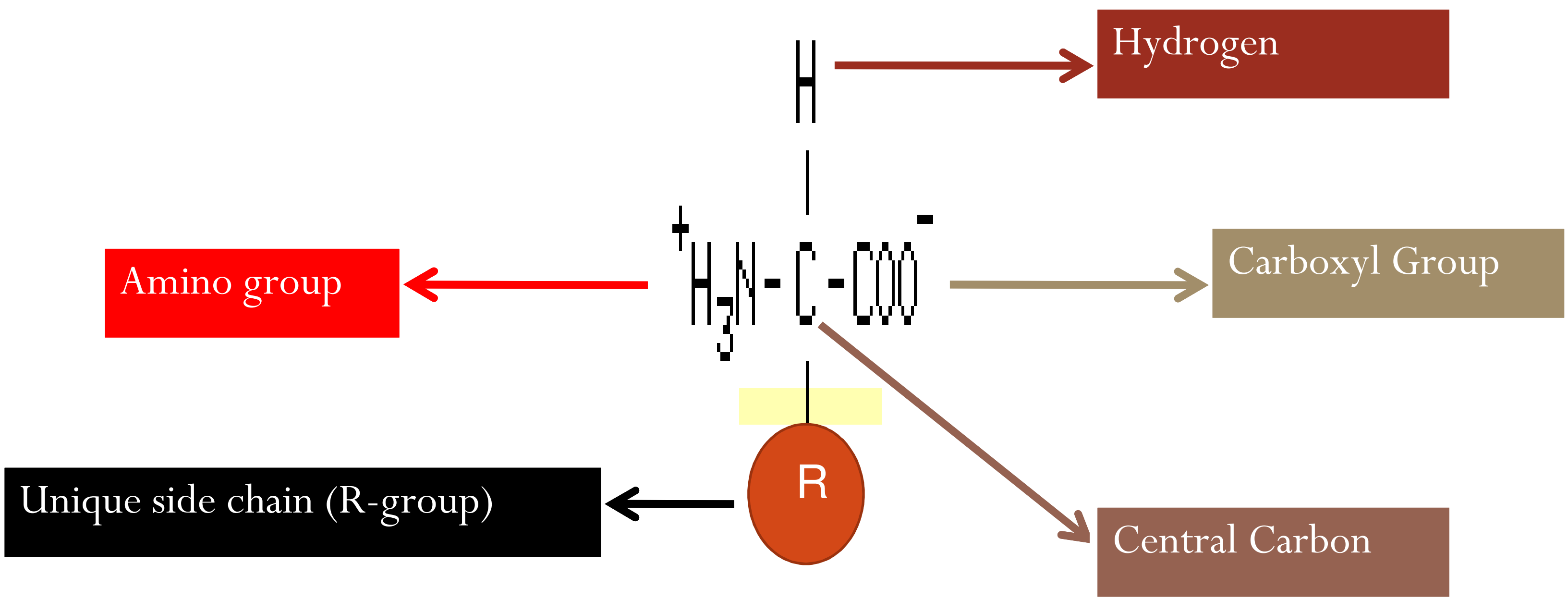
CHY2026: General Biochemistry

Unit 4:Amino Acid Chemistry

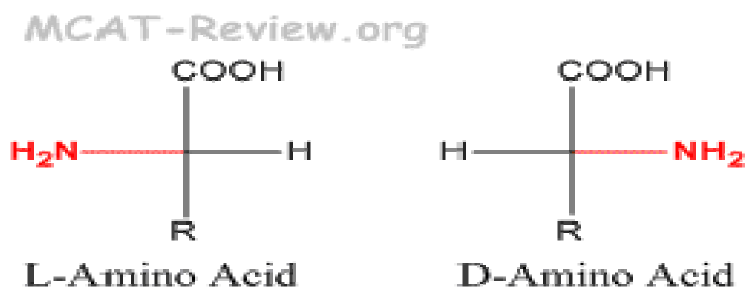


http://www.hcc.mnscu.edu/programs/dept/chem/V.27/amino_acid_structure_2.jpg


Hydrogen
Amino group
Carboxyl Group
Unique side chain (R-group)
Central Carbon
R

Amino Acid Structure

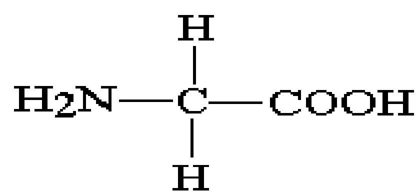
- The molecule is comprised of the elements carbon (C), hydrogen (H), oxygen (O), nitrogen (N) and small quantities of sulphur (S) – **CHONs**
- The α- carbon in all amino acids is asymmetric (except glycine)
- Because of this two optically active forms exist
- The $–NH_2$ is to the right of the –COOH: *D*-form
- The $–NH_2$ is to the left of the –COOH: *L*-form



- Only *L*- forms are used to make proteins

Amino Acid Structure

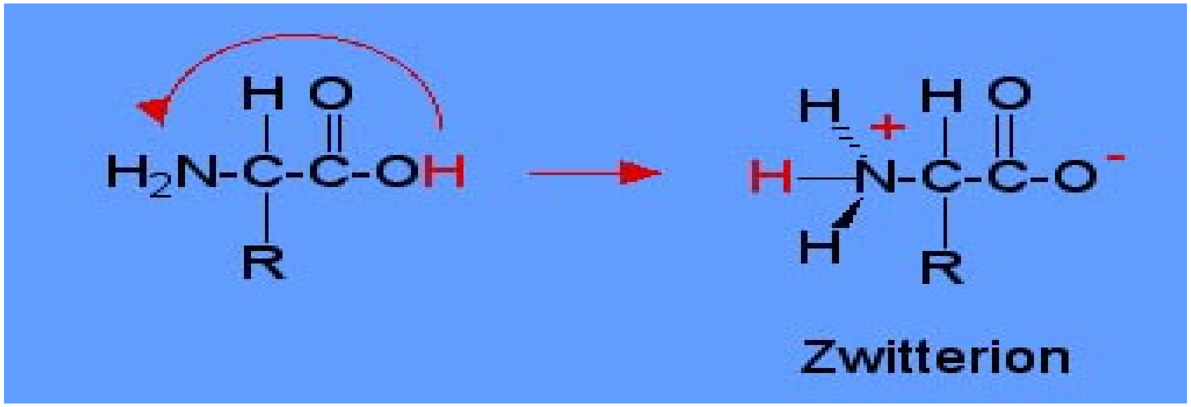
- Glycine does not contain any asymmetric carbon
- This is the simplest amino acid



glycine

Properties of Amino Acid

- Amino acids are dipolar ions **(zwitterions)** in solution
- In the dipolar form amino group becomes positively charged (NH_3^+) and the carboxylic acid becomes negatively charged (COO^-)

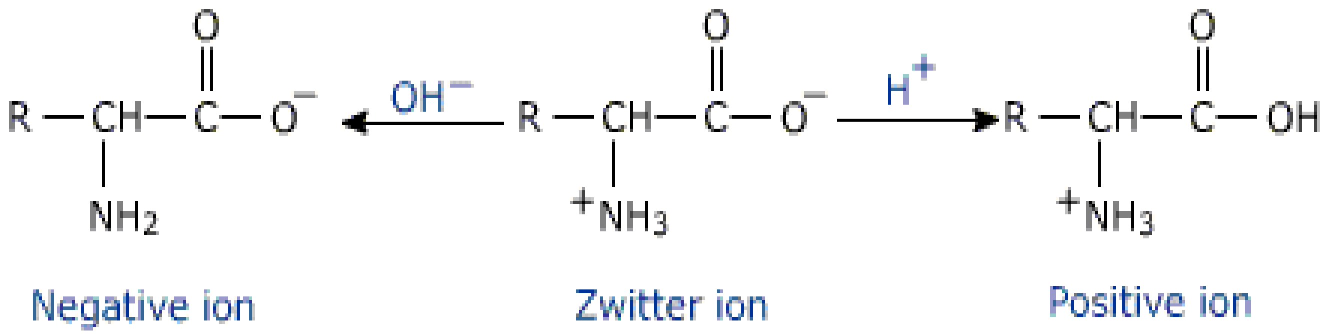


http://cyberlab.lh1.ku.ac.th/elearn/faculty/veterin/vet69/Biochemistry%20Web%20Job/amino%20and%20protein/zwitterion2.jpg

- The dipolar ion exists as a neutral ion and will have zero mobility in electrophoresis

Properties of Amino Acid

- Amino Acids are amphoteric



- There is no pH at which a.a have no ionic character
- The pH value at which the number of cations is equal that of anion is called the isoelectric point (pI)
- Every a.a have a different pI: The 3 basic a.a have higher pI and the 2 acidic a.a have lower pI
- At pH lower than the pI, the protein will have a net positive charge and as a cation will migrate towards the negative electrode (cathode)
- At pH higher than the pI, the protein will have a net negative charge and the anion will migrate towards the positive electrode (anode)

Properties of Amino Acid

- Notice that the isoelectric point depends on the amino acid structure in a predictable way

  acidic amino acids: aspartic acid (2.8), glutamic acid (3.2)

  neutral amino acids: (5.0 to 6.3)

  basic amino acids: lysine (9.7), arginine (10.8), histidine (7.6)

- *Electrophoresis uses differences in isoelectric points to separate mixtures of amino acids. A streak of the amino acid mixture is placed in the center of a layer of acrylamide gel or a piece of filter paper wet with a buffer solution. Two electrodes are placed in contact with the edges of the gel or paper, and a potential of several thousand volts is applied across the electrodes. Positively charged (cationic) amino acids are attracted to the negative electrode (the cathode), and negatively charged (anionic) amino acids are attracted to the positive electrode (the anode). An amino acid at its isoelectric point has no net charge, so it does not move.*

Amino Acids

- There are 20 amino acids that can make up proteins
- 9 essential amino acids
- 11 non essential amino acids
- **Essential amino acids** – they are not synthesized in the body and are normally required in the diet
- **Non essential amino acids** – they are synthesized *de novo* in humans

Essential	Non essential
Histidine (His)	Alanine (Ala)
Isoleucine (Ile)	Arginine (Arg)
Leucine (Leu)	Asparagine (Asn)
Lysine (Lys)	Aspartic acid (Asp)
Methionine (Met)	Cysteine (Cys)
Phenylalanine (Phe)	Glutamic acid (Glu)
Threonine (Thr)	Glutamine (Gln)
Tryptophan (Trp)	Glycine (Gly)
Valine (Val)	Proline (Pro)
	Serine (Ser)
	Tyrosine (Tyr)

Amino Acids

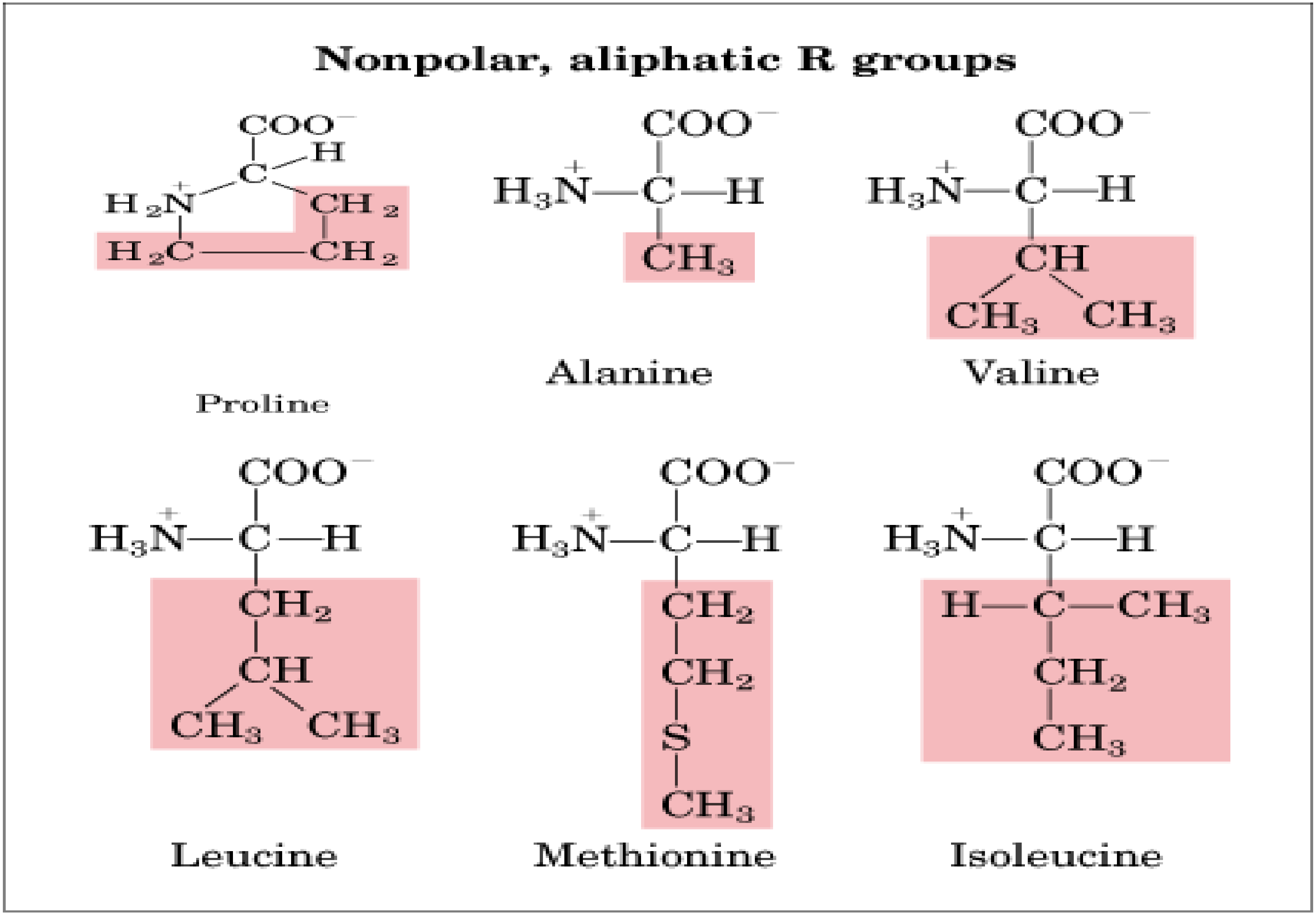
- Amino acids can be grouped based on the properties of the R group

  non polar

  polar uncharged

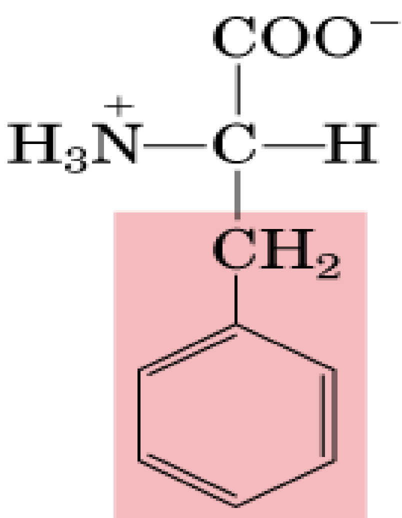
  acidic (negatively charged)

  basic (positively charged)

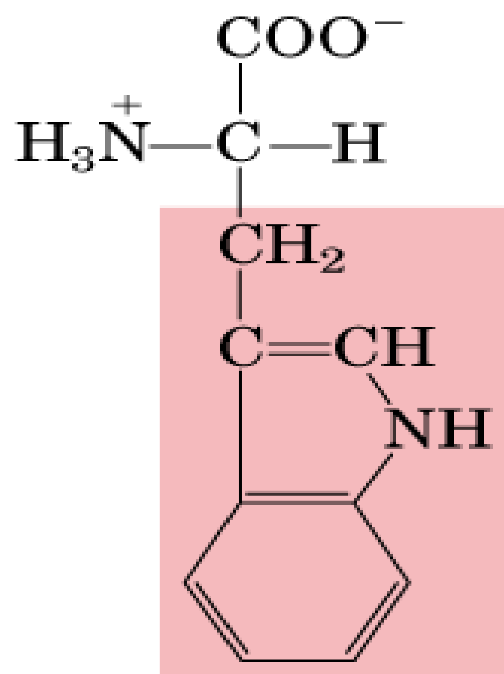


http://www.le.ac.uk/by/teach/biochemweb/images/nonpolar.gif

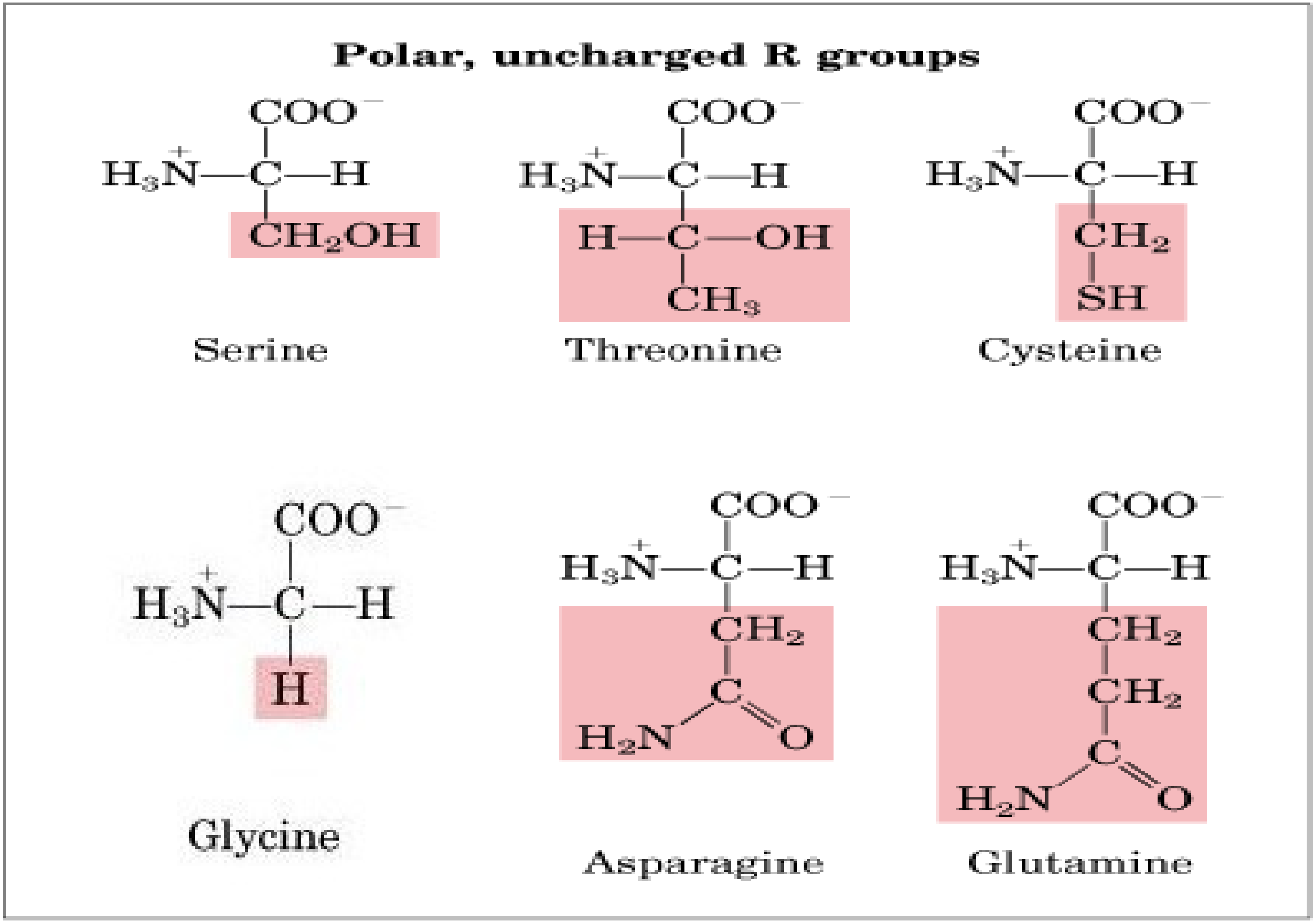
Non polar Aromatic R group



Phenylalanine

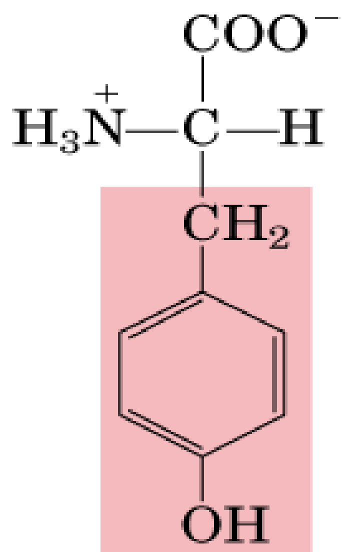


Tryptophan



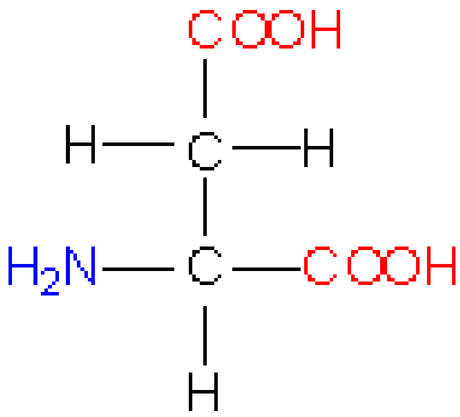
http://www.le.ac.uk/by/teach/biochemweb/images/uncharged.gif

Polar uncharged aromatic R group

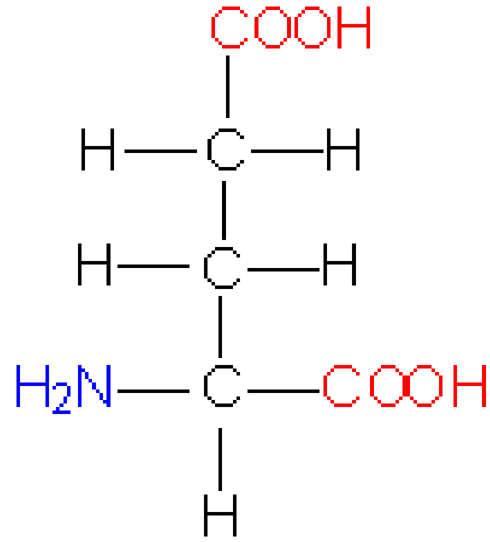


Tyrosine

Acidic R group (negatively charged R group)

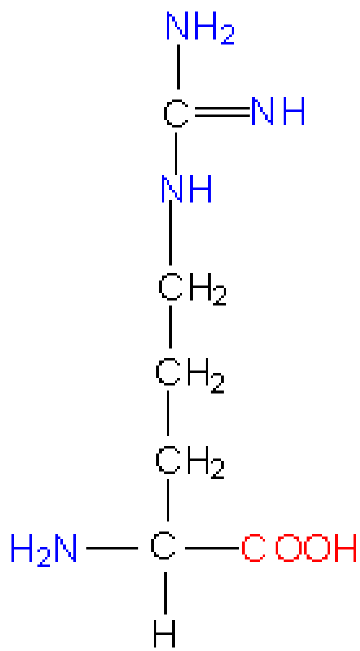


Aspartic acid

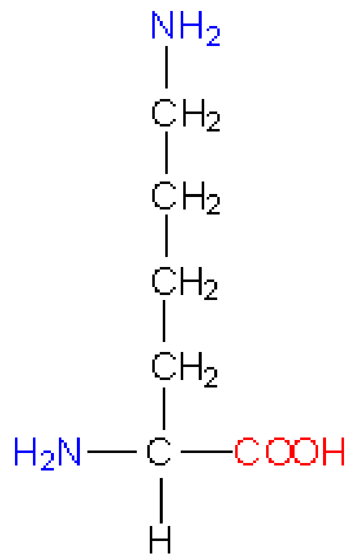


Glutamic acid

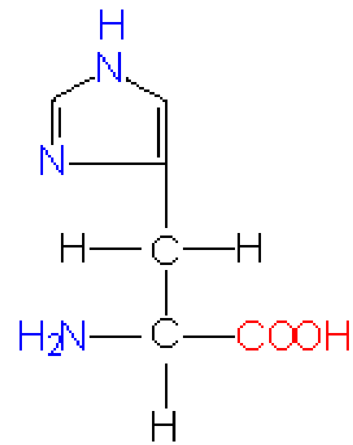
Basic R group (positively charged R group)



Arginine



Lysine



Histidine

Acid Dissociation Constant: pKa

- The dissociable α-COOH and α-NH_3^+ are responsible for the two characteristic pK values
- A third amino acid is indicative of a dissociation in the R group (pK_R)
- *(pH and pK_a are notations for proton concentration and equilibrium constant for ionization… pK_a is a measure of the tendency of a group to give up protons)*
- Ionic properties of amino acids

  α-Carboxyl group: pKa's range from 2.0 to 2.4

  α-Amino group: pKa's range from 9.0 to 9.8

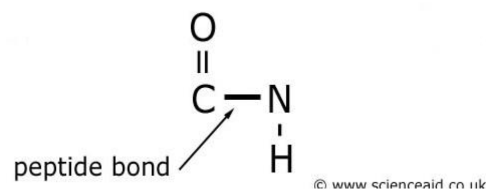
  Side chains

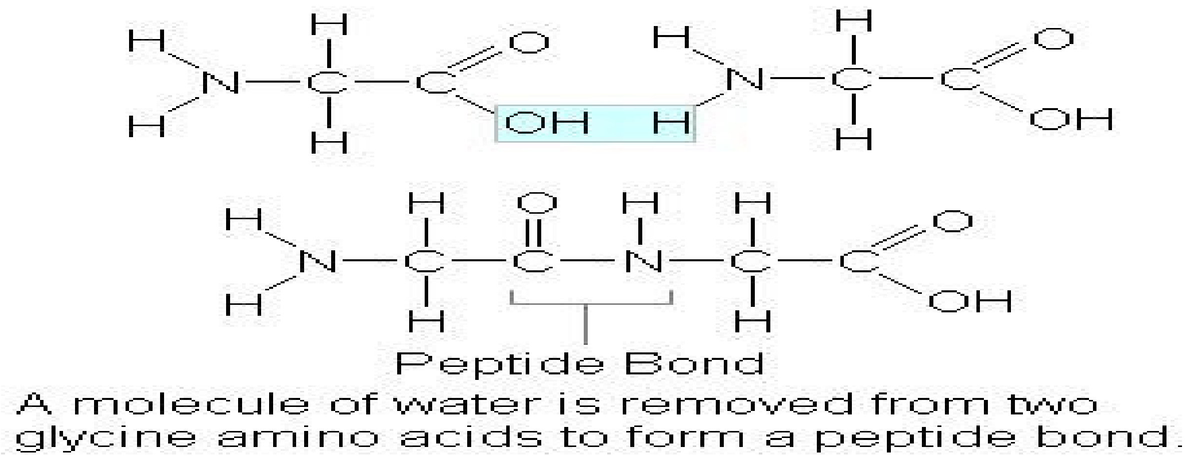
  Acidic pKa's for asp, glu

  Basic pKa's for his, arg, lys

Proteins

- Proteins are made up of amino acids linked together by peptide bonds (condensation reaction)





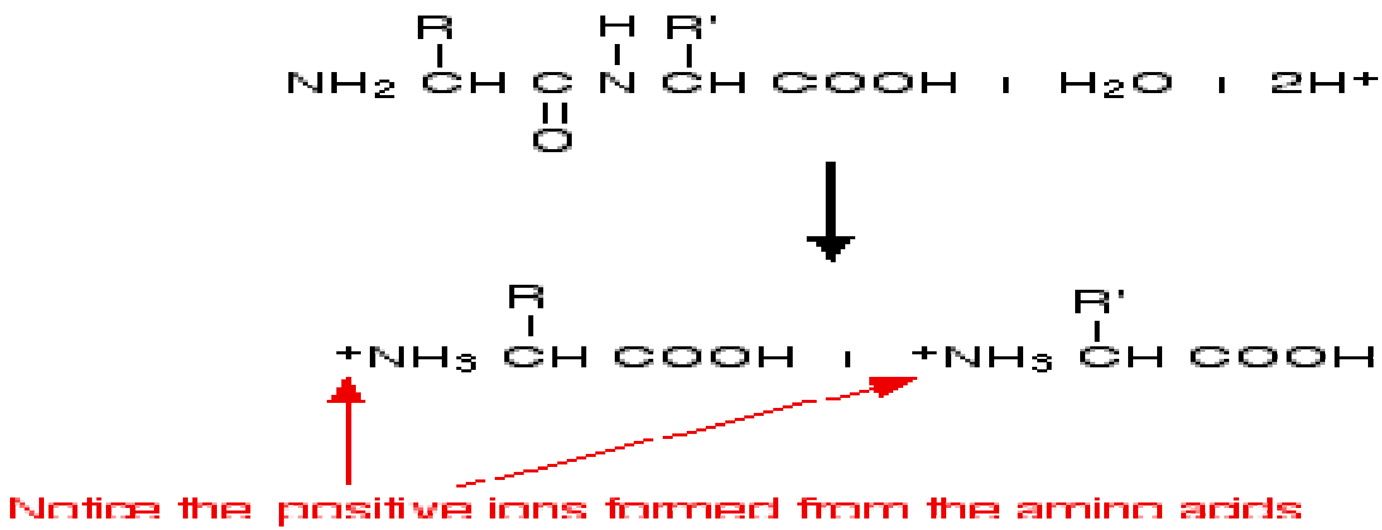
- The peptide bonds in proteins are metastable, meaning that in the presence of water they will break spontaneously, releasing 2-4 kcal mol^{-1} of free energy, but this process is extremely slow
- In living organisms, the process is facilitated by enzymes
- Living organisms also employ enzymes to form peptide bonds; this process requires free energy

Proteins

- 2 amino acids react → dipeptide
- 3 amino acids → tripeptide
- Less than 10 amino acids - oligopeptide
- 4 or more amino acids → polypeptide
- Polypeptide with a MW > 6000 or ≥ 100 amino acids → protein
- Each amino acid in a chain is termed a *residue*
- The two ends of the chain are named amino acid terminal *(N- terminal)* and carboxylic terminal *(C-terminal)* respectively
- These terminals are the only ionizable groups *(except for the side chain)* in a protein

Hydrolysis

- Proteins can be hydrolyzed (using acid or a suitable enzyme) to form individual amino acids



http://www.chemguide.co.uk/organicprops/aminoacids/hydrolprotein.gif

Biological Roles of Peptides

- Intermediates for the formation of proteins
- Antibacterial properties e.g. penicillin
- Growth factors e.g. folic acid
- Hormones
- Control oxidation-reduction potential of cell e.g. glutathione
- Increased levels in urine may be indicative of psychological disorders such as depression and schizophrenia

Industrial Importance of Peptides

- Inhibitory peptides are used in clinical research to examine the effects of peptides on the inhibition of cancer proteins and other diseases
- Peptides are used in imaging where, specific peptides in the fluorescing material help with early detection of potentially life-threatening cancers
- Peptides are used in skin care to increase the amount of collagen being produced in the skin, stimulate fibroblast growth, and stimulate the production of keratinocytes
- The zero-calorie sweetener found in many diet beverages is a synthesized peptide

Proteins

- They are the most abundant intracellular macromolecule – most of the protein mass is found in the skeletal muscle
- Proteins found in animal contains 0.5 -2.0% sulphur except for insulin which contains 3.4%

Biological Role of Proteins

- They are biological catalysts – **enzymes**
- They act as carrier molecules transporting small molecules and ions e.g. haemoglobin (protein) transports oxygen in the erythrocytes
- Responsible for the high tensile strength of the skin and bone
- Immunoregulation
- Receptor proteins help in the transmission of nerve impulses
- Major component of muscle

Protein Classification

1. **Classification based on source**

    (a) Animal protein – higher quality protein

    (b) Plant protein – low quality protein (does not mean it's a poor protein source)

2. **Classification based on shape**

    (a) Globular/Corpuscular proteins: spherical or ovoid shape, soluble in water or aqueous media. Tertiary and quaternary structures are usually associated with this class

    Further classified as:

    - Cytochrome C
    - Blood proteins: serum albumin, glycoproteins, antibodies, haemoglobin, hormones
    - Enzymes
    - Nutrient Proteins

    (b) Fibrous/Fibrillar proteins: serve in structural or protective roles

- They can stretch and later recoil e.g. collagens, elastins, keratins and fibroin

Protein Structure

- Protein structure can be grouped into four levels of organization

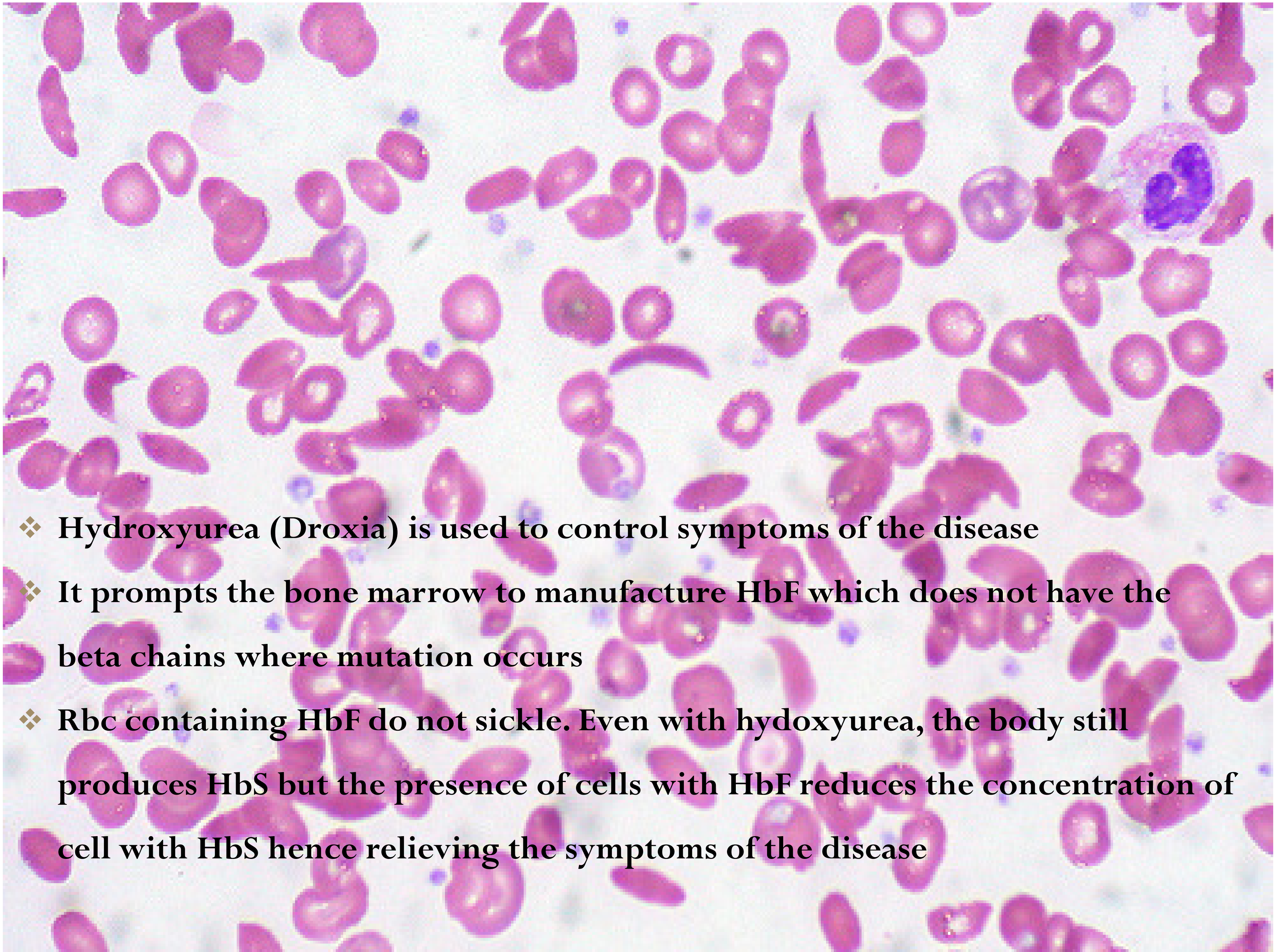
1. Primary structure – linear sequence of amino acids. Main linkage – peptide bonds

How important is the primary sequence?

- Some small change in a.a can make a big difference
- Normal Hb has 2 alpha chains and 2 beta chains

Normal Hb	-Thr-Pro-Glu-Glu-Lys-Ala
Sickle cell Hb	-Thr-Pro-Val-Glu-Lys-Ala

- Red blood cells carrying HbS behave normally when there is an ample supply of oxygen. Reduced oxygen concentration results in the rbc becoming sickle-shaped
- Sickled cells may clog capillaries
- The body's defenses destroy the clogging cells and the loss of blood cells causes anaemia



- Hydroxyurea (Droxia) is used to control symptoms of the disease
- It prompts the bone marrow to manufacture HbF which does not have the beta chains where mutation occurs
- Rbc containing HbF do not sickle. Even with hydoxyurea, the body still produces HbS but the presence of cells with HbF reduces the concentration of cell with HbS hence relieving the symptoms of the disease

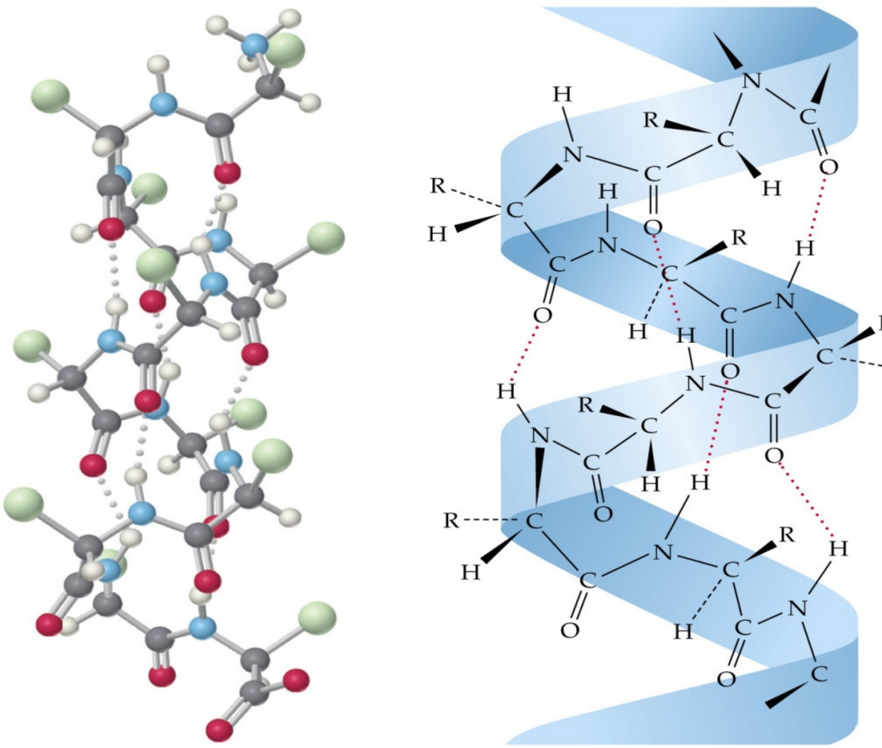
2. Secondary structure – spatial arrangement of a portion of the polypeptide. Main linkage- H bonding

(a) α- helix e.g. α-keratin

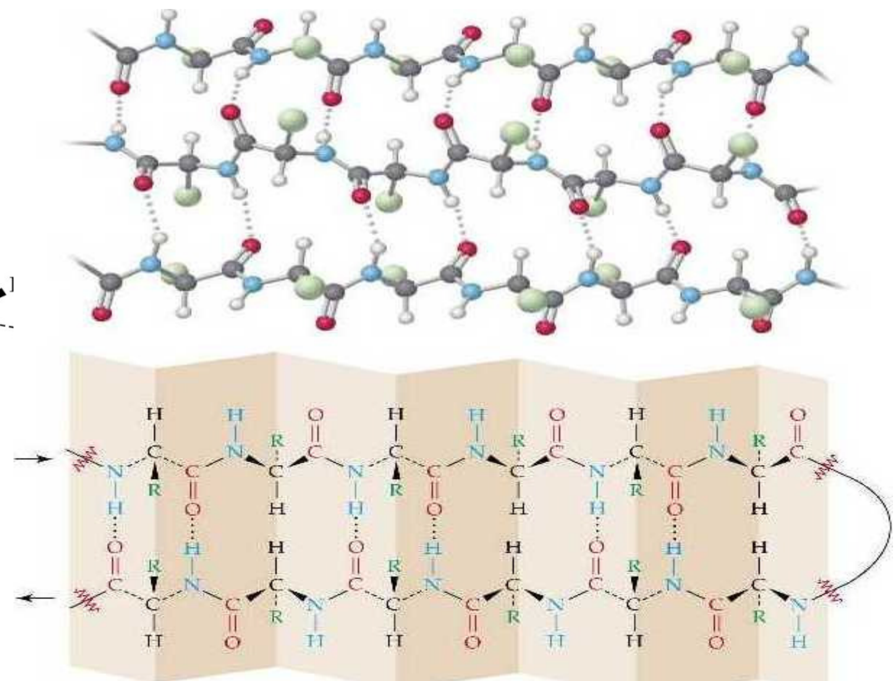
(b) β- pleated sheets e.g. silk fibroin

(c) random coil

α-keratin

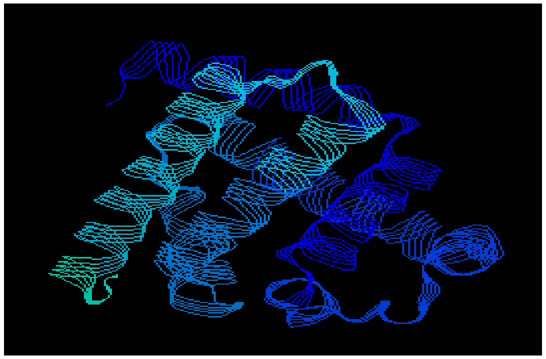


Silk fibroin



Protein Structure

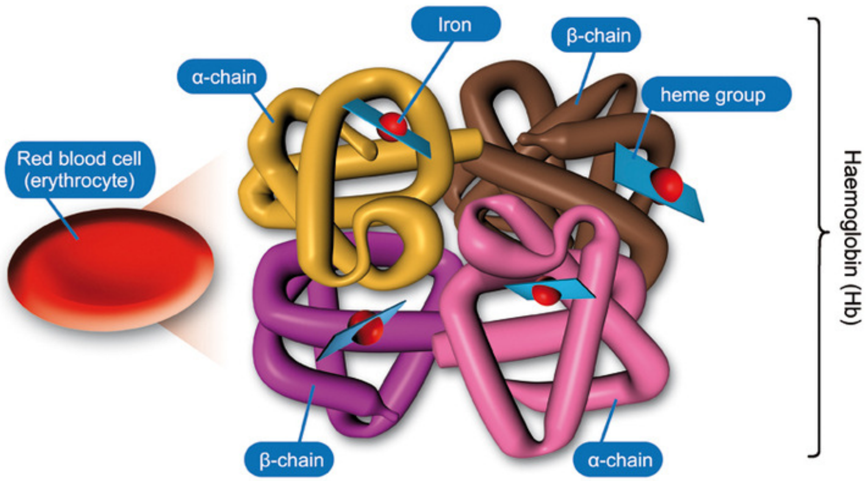
3. Tertiary structure – describe the shape of the entire polypeptide…folding of the helices of globular proteins. All the chemical bonds are found in the structure e.g. myoglobin (cardiac and red skeletal muscle)



Chaperones – a protein that helps other proteins to fold into the biologically active conformation and enables partially denatured proteins to regain their biologically active conformation

4. Quaternary structure – spatial arrangement of many polypeptide subunits…all chemical bonds are found in the structure e.g. haemoglobin (tetrameric protein)

Structure of haemoglobin



Each erythrocyte (RBC) contains ~270 million haemoglobin molecules

Haemoglobin

- In the adult human, Hb (tetramer) consists of 4 polypeptide chains (globins) – 2 identical α chains (141 residues) and 2 identical β chains (146 residues)
- Each globin chain surrounds an iron-containing heme
- It is a conjugated proteins – proteins that contains non-amino acid portion

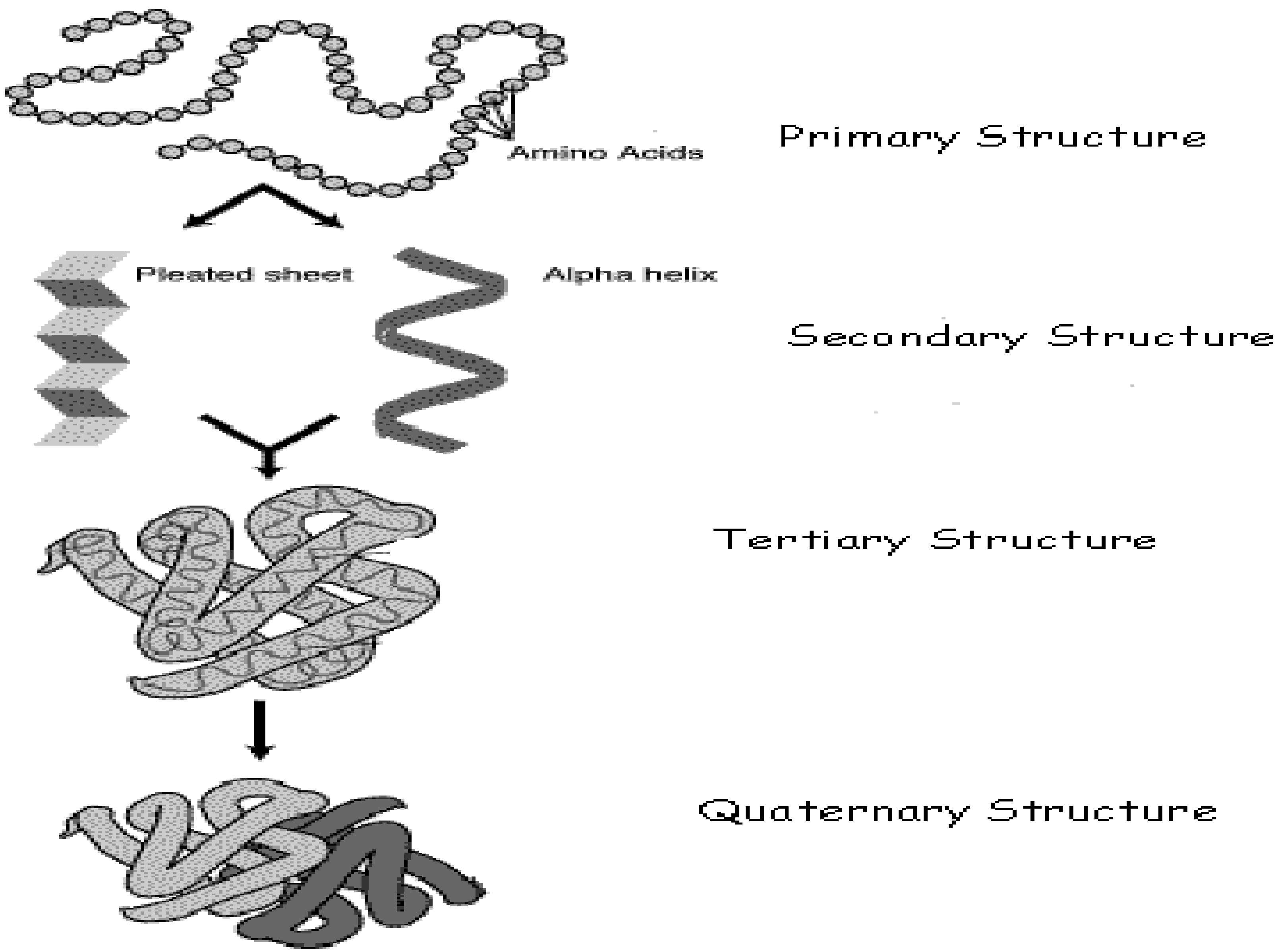
  Prosthetic group – the non-amino acid portion of a conjugated protein
- The globins are the amino acid portion and the prosthetic groups are the haem units

Myoglobin

- Myoglobin is a small oxygen heme protein found in the muscle
- Myoglobin consist of 1 polypeptide chain, 153 residues long

Comparison between Haemoglobin and Myoglobin

Haemoglobin	Myoglobin
1 Hb can bind to 4 O_2	1 myoglobin binds to 1 O_2
The binding of oxygen to Hb exhibit positive cooperativity (when 1 oxygen molecule is bound it becomes easier for the other oxygen to bind	Oxygen binding to myoglobin does not exhibit positive cooperativity
Hb functions in **oxygen transport**. It must bind strongly to oxygen and be able to release it easily	Myoglobin has the function of **oxygen storage** in muscles



http://www.contexo.info/DNA_Basics/images/proteinstructuresweb.gif

Conformation of Proteins

- Protein folding is the physical process by which a polypeptide folds into its characteristic and functional three dimensional structure from random coil
- Each protein exists as an unfolded polypeptide when translated from a sequence of mRNA to a linear chain of amino acids
- This polypeptide lacks any developed three dimensional structure
- Amino acids interact with each other to produce a well-defined three dimensional structure, the folded protein is known as the native state
- The resulting three dimensional structure is determined by the amino acid sequence
- The correct three dimensional structure is essential to function, although some parts of functional proteins may remain unfolded

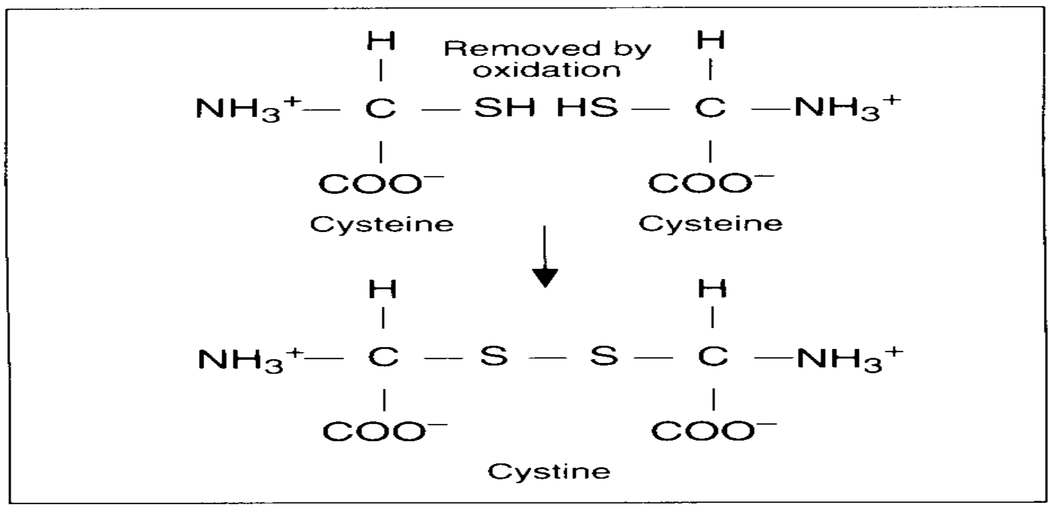
Conformation of Proteins

- Failure to fold into native structure produces inactive proteins that are usually toxic
- Several neurodegenerative and other diseases are believed to result from the accumulation of amyloid fibrills formed by *misfolded* proteins
- Many allergies are caused by the folding of the proteins, for the immune system does not produce antibodies for certain protein structures

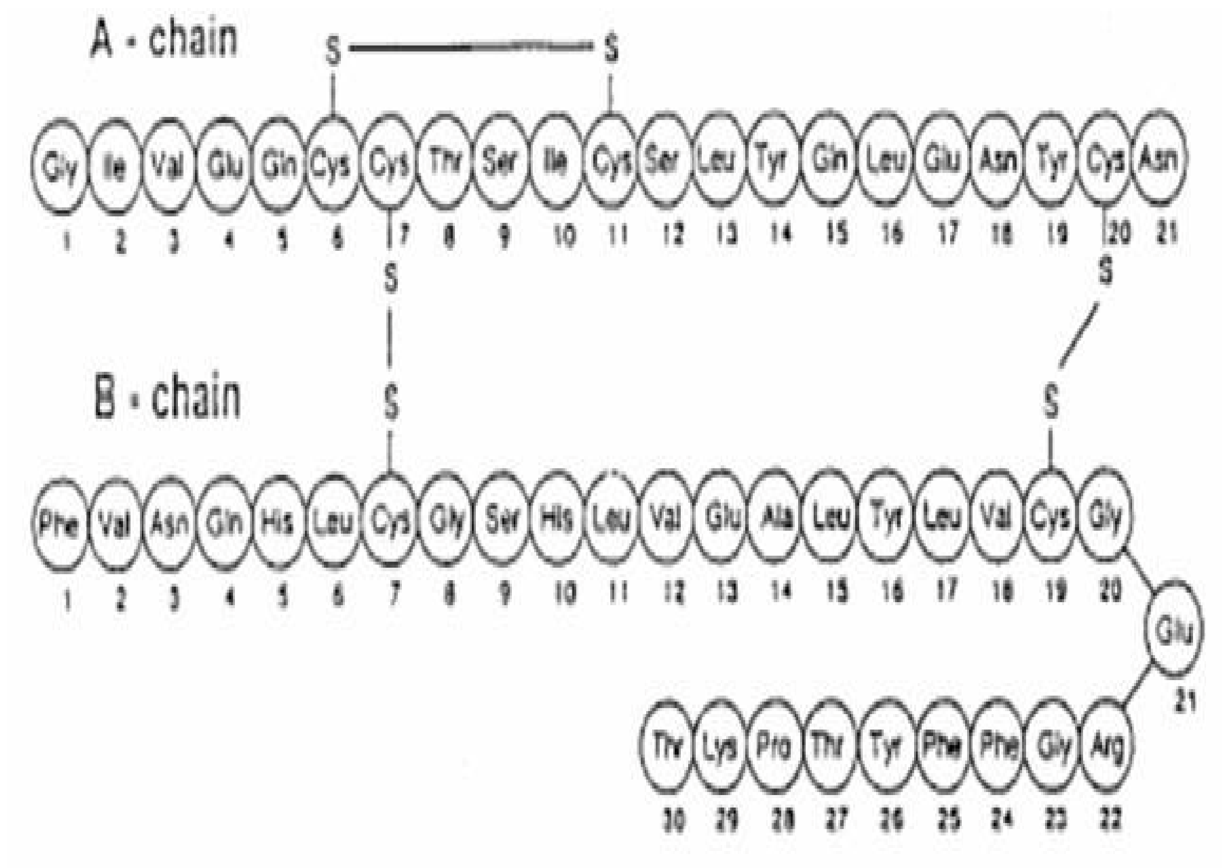
Chemical Bonds in Protein Structure

1. Primary Bond: Peptide bond…backbone of the protein chain
2. Secondary Bonds: Hold the chain in its natural configuration

(a) Disulphide Bond – formed by the oxidation of the thiol or sulphurhydryl groups (-SH) of two cysteine residues to form cystine



Disulphide linkages can occur in two cysteine molecules that are apart in a polypeptide chain. This will cause the polypeptide chain to be folded back on itself e.g. oxytocin and insulin



(c) Hydrogen Bond: This occurs when a group containing a H atom is bonded to the electronegative atoms O or N. H-bond plays a role in stabilizing some conformations of the polypeptide chain e.g. silk fibroin

3. Nonpolar/ Hydrophobic Bond formed among amino acids with hydrophobic side groups
4. Ionic/Electrostatic Bond/Salt Bridge/Salt linkage: Ions possessing similar charge repel each other…ions possessing dissimilar charge attract each other e.g. acidic and basic R groups

Ionic bonds are responsible for maintaining the folded structure of globular proteins

Denaturation

- This refers to a change in the properties of a protein resulting in a loss of biological activity
- Denaturation can be brought about by ***physical agents***

  (a) shaking

  (b) heat treatment

  (c) cooling or freezing

  (d) UV rays

  and ***chemical agents***

  (a) ionizing radiation

  (b) organic solvents

  (c) detergents

- When proteins are denatured the molecule unfolds causing disorganization of internal structure … (bonds are broken)
- Some denatured proteins cannot be brought back to their original state….however generally its a reversible process

Methods of Protein Separation

- Electrophoresis - used to separate complex mixtures of proteins and to purify the protein for subsequent applications
- Western blotting is used to identify a specific protein in a complex mixture of proteins. The technique exploits both the efficiency of SDS-PAGE to separate a mixture of proteins into distinct protein bands, and the ability of immunochemical reagents to interact specifically with a given protein antigen
- Column Chromatography can be used to fractionate proteins. The mixture of proteins in solution is passed through a column containing a porous solid matrix

  The 3 types of matrices used are:

  (1) Ion-exchange chromatography, (2) gel-filtration chromatography and (3) affinity chromatography

Protein Quantification

- Knowing the quantity of a protein after each separation step is useful in checking the progress of purification and evaluating the technique's efficiency
- The concentration of a protein can be measured by immunological techniques such as ELISA or Western Blotting
- Activity can be measured using fluorescent techniques
- Mass spectrometry is another accurate analytical technique for determining protein mass. In this technique, atoms are ionized through a machine and passed through a vacuum into the detector

Industrial Importance of Proteins

- Denatured protein - used in the formation of gelatin, which is used in many foods, in the pharmaceutical and cosmetic industries
- Collagen has been widely used in cosmetic surgery, as a healing aid for burn patients for reconstruction of bone and a wide variety of dental, orthopedic and surgical purposes
- Collagens are also used in the construction of artificial skin substitutes used in the management of severe burns
- Corn gluten is used in feed concentrates and in small amounts as extenders for commercial resins and glues
- Corn gluten is sometimes destarched by acid or enzyme hydrolysis to yield a product that is almost entirely protein and is used in paints. Sometimes it is broken down by complete hydrolysis to yield monosodium glutamate, a seasoning for food